# On the origin of albuminuria

*Kidney International* (2007) **72,** 1409; doi:10.1038/sj.ki.5002499

**To the Editor:** Russo *et al.*[1] recently reported that the normal glomerulus filters nephrotic levels of albumin, followed by rapid endocytosis into proximal tubular cells (PTCs). Using 2-photon microscopy, they found more fluorescent albumin in the tubular lumen of puromycin aminonucleoside nephrotic rats than in healthy rats, but reduced albumin quantities in the PTC cytoplasm and reduced megalin expression. Based on their findings, the authors proposed a paradigm shift, stating that not glomerular injury but impairment of albumin endocytosis by PTCs is the cause of albuminuria. Not only is this in contrast with a spectrum of studies identifying a glomerular defect[2] as the central etiological factor but also the presented data do not support such a drastic conclusion.

Besides important questions on the quantification of fluorescence signals in this study, as raised also in the accompanying commentary,[3] one could wonder where the increased intraluminal albumin in puromycin aminonucleoside rats comes from. Did the authors determine the glomerular sieving coefficient in puromycin aminonucleoside rats? In addition, the reduced albumin uptake by PTCs and associated decreased megalin expression could well be the consequence instead of the cause of albuminuria. Long-term albumin exposure reduces endocytosis in PTCs.[4] Moreover, a recent elegant study reported that high albumin levels induce apoptosis in PTCs through reduction of megalin, protein kinase B, and Bad.[5]

Thus, it seems more plausible that in puromycin aminonucleoside rats, high intraluminal albumin levels — resulting from glomerular injury — are toxic to PTCs. The study by Russo *et al.* does not provide convincing evidence that albuminuria is of tubular origin.

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# Response to 'On the origin of albuminuria'

*Kidney International* (2007) **72,** 1409–1410; doi:10.1038/sj.ki.5002537

**We thank Dr De Borst[1] for his interest in our paper. He raises the possibility that in nephrotic states such as puromycin aminonucleoside (PAN) nephrosis, increased luminal albumin due to increased permeability of the glomerular filtration barrier could be a potential cause of proximal tubule cell (PTC) toxicity, rather than a direct effect of PAN on tubule cells. While we agree that this could theoretically be a potential mechanism, we believe that evidence from the literature suggests that this may not be the case. We know that PAN nephrosis in albumin-deficient analbuminemic rats is accompanied by exactly the same type of pathology and proteinuria as normoalbuminemic rats, indicating that albumin itself is not the specific cause.[2] The relevance of albumin toxicity in PTC cell cultures to the *in vivo* situation is also problematic because the albumin concentrations used and the actual amounts of albumin exposed to the PTC *in vitro* are far in excess of those found in any pathological state *in vivo*. While we agree that concentrations of 10 mg/ml or greater do indeed induce apoptosis in PTCs *in vitro*,[3] achieving this very high concentration *in vivo* would require a glomerular sieving coefficient of 0.3, which would of course be a death sentence in terms of plasma albumin loss prior to any potential damage to the PTC.**

**We did not directly measure glomerular sieving coefficient in PAN-treated rats by two-photon microscopy, because preliminary experiments by ourselves and others[4] have indicated a good deal of heterogeneity in the PAN luminal albumin concentrations, which may be due to low glomerular filtration rate or occluded nephrons. For this reason we cannot exclude the possibility that an increased glomerular sieving coefficient did occur in our experimental conditions, and that this may have contributed to the increased tubular levels of albumin observed by two-photon microscopy. However, many *in vivo* whole animal clearance studies, have demonstrated that glomerular permeability in PAN and other nephrotic states[2,5,6] is not altered for 36 Å radii molecules the size of albumin. The available evidence clearly demonstrates that albumin glomerular transcapillary transport is governed primarily by size selectivity alone as confirmed by our two-photon measurements (which were validated by the linear plasma-dose fluorescence and the equivalence of the *in vivo* and *in vitro* plasma fluorescence). No other major restrictive forces have been identified, particularly as charge selectivity has been shown to be essentially nonexistent.[5,7–9] Therefore, we stand by our conclusions that even a normal healthy glomerulus is always leaking nephrotic levels of**

**albumin and that the proximal tubule plays a major role in preventing albumin wasting into the urine.**


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# Albumin concentration in the Bowman's capsule: Multiphoton microscopy vs micropuncture technique

*Kidney International* (2007) **72,** 1410–1411; doi:10.1038/sj.ki.5002501

**To the Editor:** In their recent report, Russo *et al.*[1] measured glomerular albumin filtration in the rat using multiphoton microscopy. They estimated that, under normal condition, albumin filtration is more that 50 times greater than that previously reported with a calculated albumin sieving coefficient (glomerular sieving coefficient) of 0.02–0.04. Assuming that normal plasma albumin concentration in glomerular capillaries is 3.0–3.5 g/dl, one can calculate an expected albumin concentration in the Bowman's capsule in the range of 600–1400 $\mu$g/ml. As pointed out also in the commentary of Gekle,[2] these values are in great contrast to those previously reported in the literature and obtained by micropuncture technique. Owing to technical difficulties, there are only a few sets of data on albumin ultrafiltrate concentration in the literature, and among them the most careful evaluation is considered that of Tojo and Endou.[3] Using a sophisticated double pipette technique, they estimated a mean value of Bowman's capsule albumin concentration of 23 $\mu$g/ml, and a corresponding glomerular sieving coefficient of 0.00062. Recently, Lazzara and Deen[4] reviewed available evidence for glomerular permselective function and concluded that albumin concentration in glomerular ultrafiltrate in normal condition must range from 20 to 30 $\mu$g/ml. Thus, the debate on the effective amount of glomerular albumin filtration is still open.[2] This is an important issue, since the filtered proteins across the glomerular membrane are involved in the mechanisms leading to kidney disease progression.[5] In addition, the beneficial effect of angiotensin antagonism in proteinuric renal diseases is linked to amelioration of glomerular membrane permselective properties.[6]

In the past, we performed a series of micropuncture experiments in non-proteinuric female MWF rats, with surface glomeruli, to collect ultrafiltrate from the Bowman's capsule (using an oil drop to stop proximal tubule fluid flow). In that occasion, we measured albumin concentration by a sensitive enzyme-linked immunoassay. Although we performed these measurements in only three animals, we believe that they can be of interest in the actual debate on the effective amount of albumin filtration at glomerular level. From a total number of 12 collections of Bowman's capsule fluid, lasting 10 min in average, we calculated a mean albumin filtration of $0.34 \pm 0.28$ pg/min and a concentration of $10.7 \pm 4.9$ $\mu$g/ml (as shown in Figure 1). Our observation would support the hypothesis of a low albumin concentration in the Bowman's capsule and in the early proximal tubule, in line with previous micropuncture measurements.[3] In addition, as pointed out in the commentary of Gekle,[2] our data would indicate that the observation of Russo *et al.*[1] may

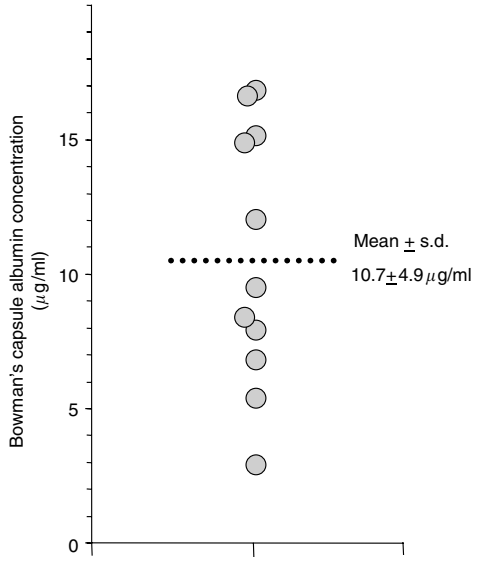


**Figure 1 | Values of albumin concentration in the Bowman's capsule of female Munich-Wistar rats as determined by micropuncture technique.**